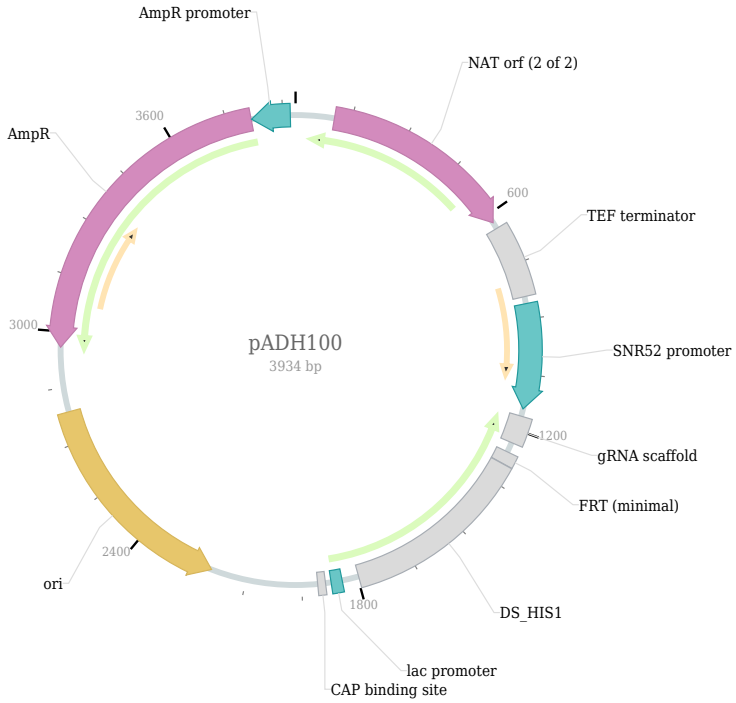
pADH100

载体编号 Vector ID	V014668
抗性 Antibiotic Resistance	Ampicillin
长度 Length	3934 bp
载体类型 Vector Type	Genome editing
宿主 Host	Yeast

产品信息

复制子 Replication Origin	ori
筛选标记 Selection Marker	NAT
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
备注 Comment	pADH99 and pADH100 are the two-component plasmids of the HIS-FLP recyclable CRISPR-Cas9 genome editing toolkit for Candida albicans. They must be co-transformed as linearized DNA fragments to achieve markerless, iterative gene manipulation in nourseothricin-sensitive strains. pADH100 is the blank sgRNA backbone vector with the second half of split NAT marker (NAT 2 of 2). Core elements: DS_HIS1 downstream homology arm, another FRT site, pSNR52 sgRNA expression scaffold, partial NAT fragment, and universal sgRNA cloning/stitching template. pADH100 functions: Provides 3’ homologous sequence for HIS1 locus integration; accepts target-specific 20 nt guide sequence via cloning-free stitching PCR or soCPEC; completes functional NAT marker when recombined with pADH99’s NAT segment. Linearized pADH99 and pADH100 co-transform C. albicans. Split NAT fragments recombine to form a full NAT cassette for nourseothricin selection. US_HIS1 and DS_HIS1 direct the whole CRISPR cassette to integrate at the endogenous HIS1 locus. Cas9-sgRNA complex creates DNA double-strand breaks at target genes, stimulating HDR-mediated homozygous knockout, point mutation or gene addback without permanent markers at edited loci. Maltose-induced FLP recombinase removes all integrated Cas9, sgRNA and NAT sequences, leaving only a single FRT scar at HIS1. The recovered nourseothricin-sensitive strain can undergo multiple rounds of CRISPR editing.

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.